HIV

Immune correlates of the Thai RV144 HIV vaccine regimen in South Africa

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One of the most successful HIV vaccines to date, the RV144 vaccine tested in Thailand, demonstrated correlates of protection including cross-clade V1V2 immunoglobulin G (IgG) breadth, Env-specific CD4$^+$ T cell polyfunctionality, and antibody-dependent cellular cytotoxicity (ADCC) in vaccinees with low IgA binding. The HIV Vaccine Trials Network (HVTN) 097 trial evaluated this vaccine regimen in South Africa, where clade C HIV-1 predominates. We compared cellular and humoral responses at peak and durability immunogenicity time points in HVTN 097 and RV144 vaccinee samples, and evaluated vaccine-matched and cross-clade immune responses. At peak immunogenicity, HVTN 097 vaccinees exhibited significantly higher cellular and humoral immune responses than RV144 vaccinees. CD4$^+$ T cell responses were more frequent in HVTN 097 irrespective of age and sex, and CD4$^+$ T cell Env-specific functionality scores were higher in HVTN 097. Env-specific CD40L$^+$ CD4$^+$ T cells were more common in HVTN 097, with individuals having this pattern of expression demonstrating higher median antibody responses to HIV-1 Env. IgG and IgG3 binding antibody rates and response magnitude to gp120 vaccine– and V1V2 vaccine–matched antigens were higher or comparable in HVTN 097 than in RV144 ADCC, and ADCP functional antibody responses were elicited in HVTN 097. Env-specific IgG and CD4$^+$ Env responses declined significantly over time in both trials. Overall, cross-clade immune responses associated with protection were better than expected in South Africa, suggesting wider applicability of this regimen.

INTRODUCTION

The need for a vaccine to prevent HIV-1 acquisition remains evident, especially in the most burdened region of southern Africa, which is dominated by clade C infections. Although the rollout of treatment and prevention programs has contributed to efforts to stem the epidemic, in 2017 alone, there were an estimated 800,000 new infections and 19.6 million people living with HIV in east and southern Africa (*1*). In the Republic of South Africa (RSA), the country with the largest HIV burden, the epidemic is generalized with heterosexual intercourse being the main mode of transmission.

RV144 was the first vaccine clinical trial to demonstrate any efficacy for preventing HIV-1 acquisition (*2*). Although estimated vaccine efficacy was as high as 60% at month 12, it waned thereafter to 31.2% by month 42 (*3*). Conducted in Thailand, with the clade B HIV-1 strain, CRF01_AE predominating, RV144 evaluated a heterologous prime-boost combination vaccination regimen. Four injections (months 0, 1, 3, and 6) were given of ALVAC-HIV (vCP1521), a canarypox vector expressing envelope (Env) (clade E), group-specific antigen (Gag) (clade B), and protease (Pro) (clade B). In addition, two booster injections (months 3 and 6) were administered of alum-adjuvanted AIDSVAX B/E, a bivalent HIV glycoprotein 120 (gp120). The vaccine regimen induced HIV-specific humoral and cellular immune responses, some of which were found to be associated with reduced HIV infection risk, and included the following: the binding of plasma immunoglobulin G (IgG) antibodies to the variable 1 and 2 (V1V2) regions of gp120, the binding of IgA antibodies to Env, the avidity of IgG antibodies for Env in vaccinees with low IgA, antibody-dependent cellular cytotoxicity (ADCC) in vaccinees with low IgA, and the magnitude and polyfunctionality of Env-specific CD4$^+$ T cells (*4*–*8*). Despite evidence of vaccine efficacy, neutralizing antibodies against circulating tier 2 HIV-1 strains from Thailand were undetectable in the RV144 trial, suggesting that the modest efficacy was largely attributed to non-neutralizing antibody effector functions (*9*–*13*). In addition, virus sequence analyses and host genetic studies of RV144 revealed the interplay of vaccine-elicited responses, infecting viruses, and host factors. A genomic sieve analysis comparing breakthrough HIV-1 sequences between the infected vaccine and infected placebo groups, focusing on the V1V2 region of Env, identified two sites in the V2 loop associated with efficacy at amino acid positions 169 and 181. (*14*). A follow-up sieve analysis also identified potential immune

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pressure in the V3 loop of the HIV-1 Env (*15*). Host genetic analyses identified associations of human leukocyte antigen (HLA) (*16*, *17*) and FcγR polymorphisms (*18*) with immune response correlates of risk and/or vaccine efficacy, suggesting that host factors may influence vaccine immunogenicity and efficacy.

All of these studies investigating potential correlates of vaccine efficacy in RV144 involved a retrospective evaluation of HIV-infected and uninfected persons who received the vaccine (i.e., case-control studies) or genetic sieve analyses comparing breakthrough HIV infections between vaccine and placebo recipients. To evaluate prospectively whether these same immune response correlates of risk could be elicited in South Africans, we conducted a study immunizing with the RV144 regimen containing clades B and E inserts in RSA where clade C dominates. In particular, we compared the magnitude and frequency of responses seen in South Africans to the Thai RV144 participants, as it pertains to the correlates of infection risk and potential cross-clade immune responses associated with these correlates. This study was a precursor to an adapted regimen, involving the subtype C ALVAC–HIV-1 and bivalent subtype C gp120/MF59 HIV-1 vaccine regimen HVTN (HIV Vaccine Trials Network) 100, conducted in RSA to inform the advancement to efficacy testing (*19*). Our results provide critical insights about the potential extension of this vaccine approach to other regions of the world as well as the identification of non-neutralizing functional antibodies that are elicited by this vaccine regimen.

RESULTS

Participant baseline characteristics

One hundred participants were enrolled into HVTN 097, a randomized controlled double-blind study, between 18 June 2013 and 12 December 2013 at three sites in RSA, with 91 participants receiving all four vaccinations (Fig. 1). Of the enrolled participants, 51% were male, 100% were Black African, and median age was 21.5 years, with an interquartile range of 20 to 25 years. Vaccination was discontinued in four participants, two of which were due to pregnancy. Table 1 shows the distributions of age and sex for HVTN 097 participants, similar to those in RV144 whose samples were selected for comparison. The vaccine was safe and well tolerated (fig. S1), with the side effect profile being equivalent to RV144 (*2*).

T cell responses

The vaccine regimen predominantly induced $CD4^+$ T cells directed to HIV-1 Env at the peak immunogenicity time point (2 weeks after second ALVAC/AIDSVAX vaccination), as measured by expression of interleukin-2 (IL-2) and/or interferon-γ (IFN-γ) (table S1). The Env-specific $CD4^+$ T cell response rate was significantly higher in HVTN 097 than in RV144 vaccine recipients (RV144 = 36.4%; HVTN 097 = 51.9%, $P = 0.043$), albeit overall magnitudes of the Env-specific $CD4^+$ T cell frequencies among responders were similar in both trials ($P = 0.401$) (Fig. 2A and table S1). Env-specific $CD8^{+5}$ T cell response rates, though rarely detectable in both trials, when present,

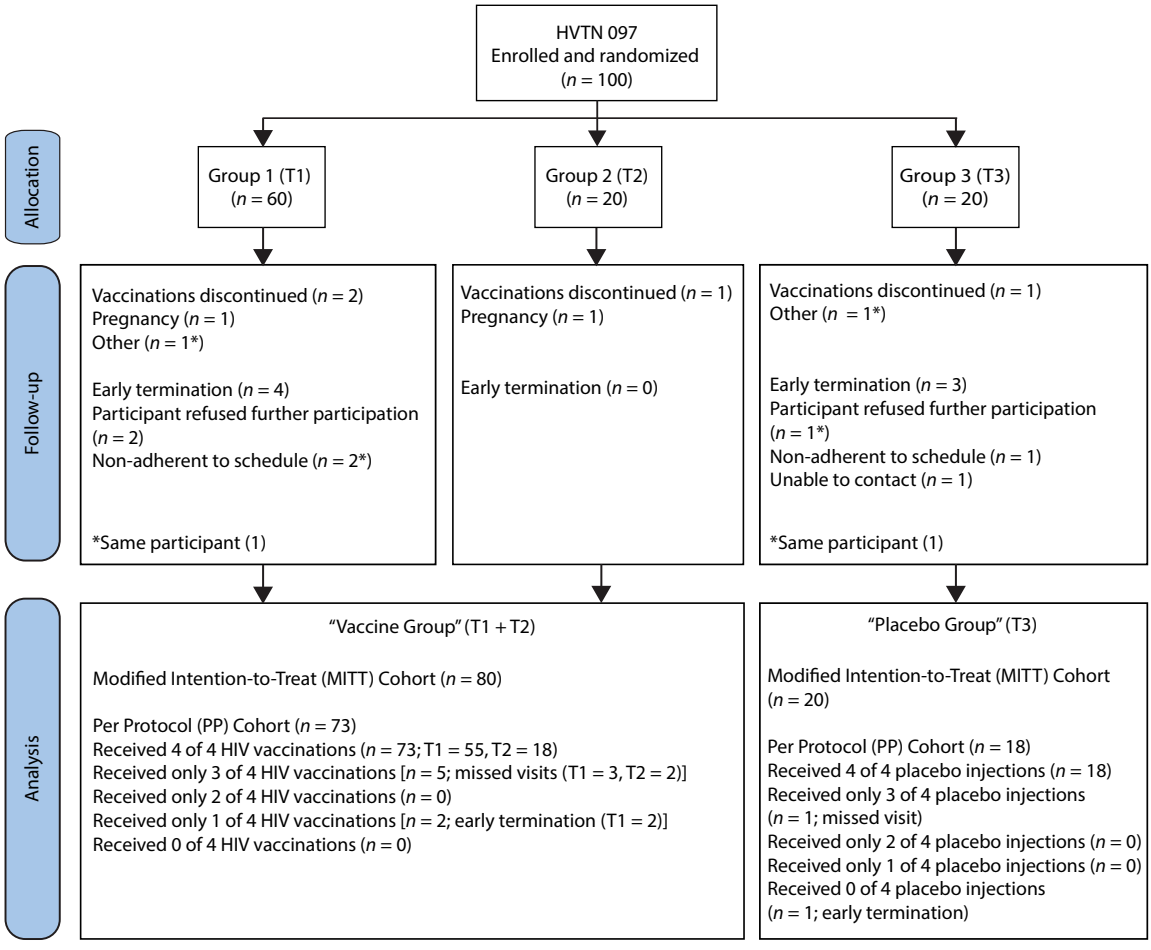

Fig. 1. CONSORT diagram.

Table 1. Demographics of participants in HVTN 097 and RV144. BMI was not measured in RV144. NA, not applicable.

		Modified intention to treat cohort			Per-protocol cohort*		
Measure		**HVTN 097**	**RV144 contemporaneous cohort (2015)†**	**RV144 case-control study (2010)‡**	**HVTN 097**	**RV144 contemporaneous cohort (2015)†**	**RV144 case-control study (2010)‡**
Treatment	Placebo	20	24	20	18	24	20
	Vaccine	80	212	205	73	201	195
Age (years)	18–20	37 (37%)	66 (28%)	52 (23.1%)	34 (37.4%)	60 (26.7%)	47 (21.9%)
	21–25	41 (41%)	112 (47.5%)	122 (54.2%)	37 (40.7%)	111 (49.3%)	118 (54.9%)
	≥26	22 (22%)	58 (24.6%)	51 (22.7%)	20 (22%)	54 (24%)	50 (23.3%)
Sex	Female	49 (49%)	98 (41.5%)	85 (37.8%)	42 (46.2%)	91 (40.4%)	75 (34.9%)
	Male	51 (51%)	138 (58.5%)	140 (62.2%)	49 (53.8%)	134 (59.6%)	140 (65.1%)
BMI	<25	66 (66%)	NA	NA	61 (67%)	NA	NA
	25–30	19 (19%)	NA	NA	18 (19.8%)	NA	NA
	≥31	15 (15%)	NA	NA	12 (13.2%)	NA	NA

*Per-protocol for HVTN 097 is defined as receiving all four HIV vaccinations, regardless of tetanus and HBV vaccination status. †RV144—Thai subjects enrolled in RV144 selected in 2015 matched on sex and vaccine schedule to South Africans enrolled in HVTN 100. The 2015 cohort was used for the main analyses (BAMA, intracellular cytokine staining). ‡RV144—Original case-control cohort selected in 2010 used for the neutralizing antibody comparison.

were significantly higher in HVTN 097 ($P = 0.031$) (table S1). HIV-1 Gag-specific $CD4^+$ and $CD8^+$ T cell response rates were very low and not statistically different between HVTN 097 (3.8%) and RV144 (2.5%), as demonstrated from samples derived from a cohort of uninfected RV144 vaccinees ($n = 40$) selected in 2010 (*20*). COMPASS analysis of the Env-specific $CD4^+$ T cell single-cell functionality and polyfunctionality scores revealed that South African participants in HVTN 097 had a higher functionality score as compared to the Thai participants in RV144 ($P = 0.038$) (Fig. 2B). The heatmaps in Fig. 2C illustrate the functional profiles induced in each study including the expression of CD40L (CD154), IFN-γ, tumor necrosis factor–α (TNF-α), IL-2, and IL-4. The HVTN 097 trial showed significantly higher response rates for CD40L (59.3% for HVTN 097 versus 33.7% for RV144, $P < 0.001$) and for IFN-γ (42.6% in HVTN 097 versus 19.5% in RV144, $P = 0.001$).

Because of concerns from a previous vaccine trial conducted in RSA demonstrating that high body mass index (BMI) was associated with reduced vaccine-induced T cell responses (*21*), we stratified the groups by BMI. In this study, higher BMI was not associated with the $CD4^+$ T cell response rate ($P = 0.153$) or the magnitude of this response ($P = 0.712$). Notably, $CD4^+$ T cell responses were detected in 100% of vaccine recipients with BMI >30, compared to 53.3 and 45.7% with BMI 25 to 30 and <25, respectively (differences not statistically significant between all three groups) (Fig. 3A). There was no difference in Env-specific $CD4^+$ T cell responses between the trials when divided by sex (Fig. 3B). The higher response rate in HVTN 097 versus RV144 effect was consistent across age groups; the 21- to 25-year-old age group showed the largest difference in response rate between South African volunteers (56%) compared to Thai volunteers (31.9%) ($P = 0.036$; Fig. 3C).

By 6 months after second ALVAC/AIDSVAX vaccination (durability time point), the frequency of circulating 92TH023-Env specific $CD4^+$ T cell responses among vaccine recipients in both studies had declined significantly when assessed by prevalence and magnitude; response rate dropped from 70.8 to 36.1% in HVTN 097 ($P < 0.001$, Table 2) and from 36.1 to 27.5% in the $n = 40$ uninfected vaccinees selected from RV144 samples in 2010 ($P < 0.001$) (*20*).

Humoral responses

The anti-gp120 IgG binding antibody response rates in vaccinees were close to 100% for all participants in both RV144 and HVTN 097 but were of higher magnitude in HVTN 097 than in RV144 (Fig. 4). Both South African and Thai participants generated cross-clade (clades AE, B, and C) antibody responses as measured by the binding antibody multiplex assay (BAMA). South Africans exhibited a higher prevalence and magnitude of antibody responses to a panel of clade C antigens to both gp120 and gp140 (Fig. 4C). IgG antibodies recognizing the V1V2 region, the primary correlate of decreased HIV-1 risk in RV144, were also significantly higher in HVTN 097 ($P = 0.004$ comparing magnitude among positive responders to any V1V2) as can be seen in Fig. 5A, fig. S2, and table S3. Significantly higher titers of V1V2 antibodies to a wide variety of clade B and clade C V1V2 antigens were seen in HVTN 097 versus RV144 participants. This included titers to the CaseA2_V1V2.B antigen, the primary correlate of reduced risk in RV144 (Figs. 4C and 5A). Overall, the magnitude-breadth curves to a diverse panel of clade C isolates were higher in HVTN 097 versus RV144 participants (Fig. 5B). Linear epitope analyses of the C1 to V2 region of Env revealed a very high prevalence of epitope-specific responses to the TH023.AE and A244.AE vaccine strains in HVTN 097 recipients (Fig. 5C). In HVTN 097, we observed a 100% response rate to the A244 linear V2 epitope that correlated with lower risk in RV144 (*22*). This increased response to both vaccine immunogens and cross-clade responses was also seen in the IgG3 responses. IgG3 binding antibody responses to both gp120 and V1V2 antigens were also higher or comparable in prevalence and magnitude among HVTN 097 participants (Fig. 5D, table S3, and fig. S3). This pattern of greater or comparable IgG or IgG3 antibody responses in HVTN 097 versus RV144 was observed consistently within sex and age subgroups and across panels of gp120 and V1V2 antigens evaluated (table S4). We looked at the relationship between BMI and immune responses in

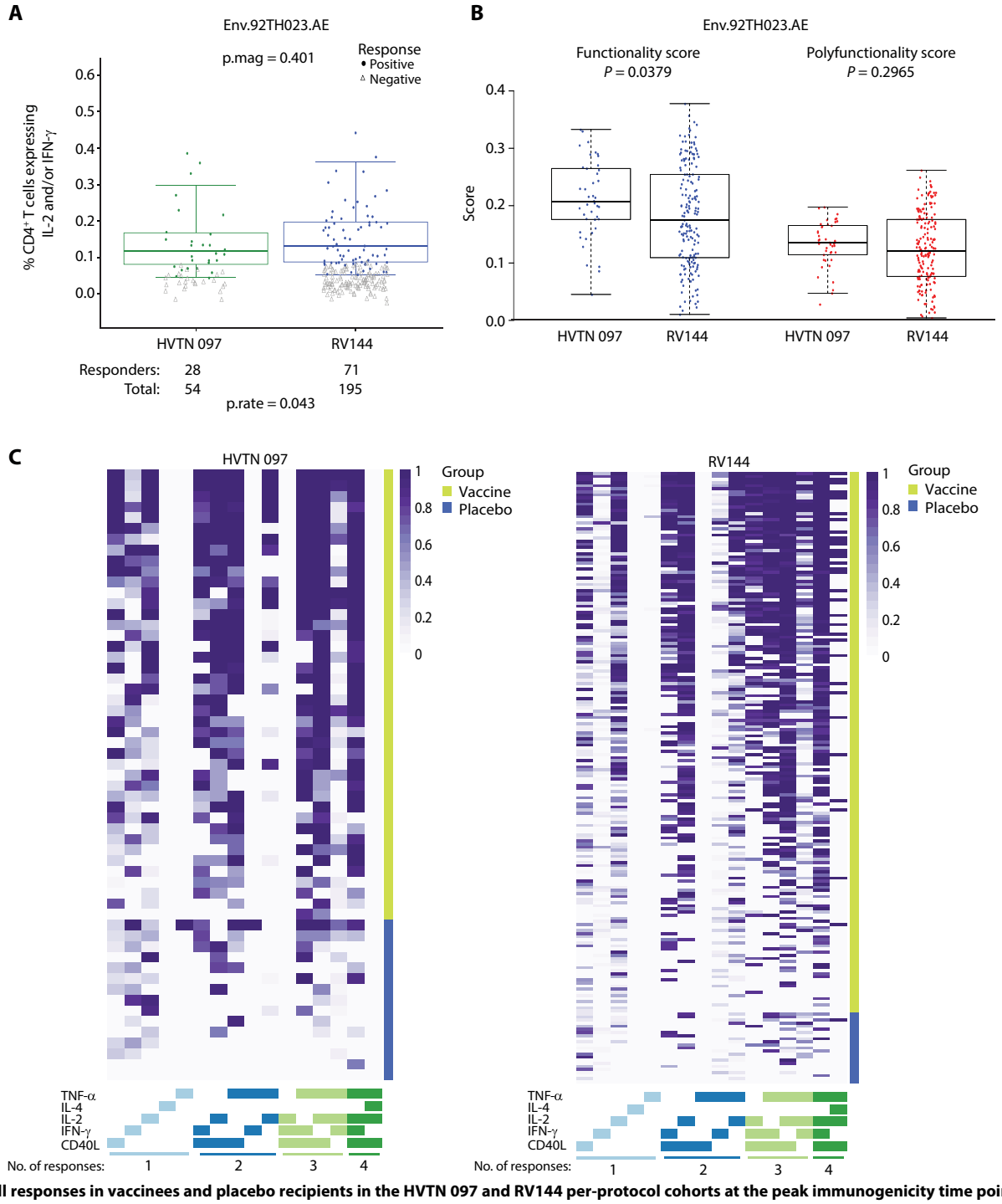

Fig. 2. T cell responses in vaccinees and placebo recipients in the HVTN 097 and RV144 per-protocol cohorts at the peak immunogenicity time point. T cell responses were measured by intracellular cytokine staining. (**A**) 92TH023-Env–specific IL-2 and/or IFN-γ $CD4^+$ T cell responses among vaccinees in the HVTN 097 and RV144 per-protocol cohorts. Boxplots are based on positive responders only, with negative responders shown as gray triangles and response rates above the boxes. A response is considered positive if the number of $CD4^+$ or $CD8^+$ T cells positive for IL-2 and/or IFN-γ is higher in the peptide-stimulated samples than in the negative control samples for at least one peptide pool (as determined by one-sided Fisher's exact test, $P < 0.00001$ after a discrete Bonferroni adjustment for testing against multiple peptide pools). Fisher's exact test *P* values comparing response rates (p.rate) and Wilcoxon rank sum test *P* values comparing magnitudes (p.mag) among positive responders between HVTN 097 and RV144 vaccine recipients are provided. (**B**) Functionality and polyfunctionality scores of 92TH023-Env–specific $CD4^+$ T cell subsets among vaccinees in the HVTN 097 and RV144 per-protocol cohorts. *P* values compare functionality or polyfunctionality scores between HVTN 097 and RV144 vaccinees. (**C**) Heatmap of COMPASS posterior probabilities for 92TH023-Env–specific $CD4^+$ T cell responses among vaccine and placebo recipients in the HVTN 097 and RV144 per-protocol cohorts. White indicates that the cytokine subset is not expressed, and purple indicates that it is expressed, ordered by degree of functionality. Rows correspond to participants, ordered by treatment group and by functionality score within each group. Each cell shows the probability [ranging from white (zero) to purple (one)] that the corresponding cell subset (column) demonstrates an antigen-specific response in the corresponding participant (row).

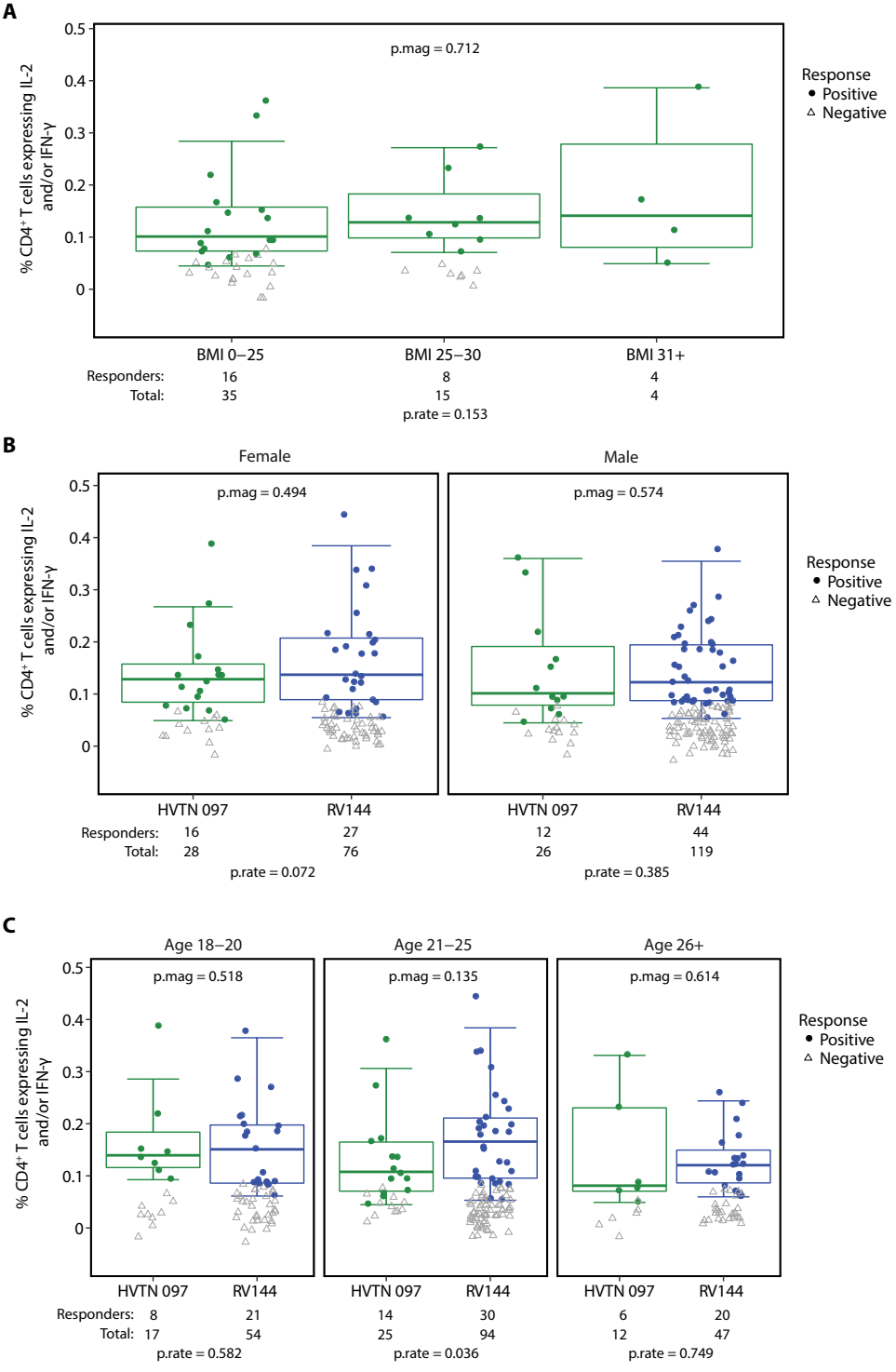

Fig. 3. IL-2 and/or IFN-γ CD4⁺ T cell responses to Env among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 at the peak immunogenicity time point. (**A**) Stratified by BMI. (**B**) Stratified by sex. (**C**) Stratified by age.

HVTN 097 vaccine recipient positive responders and observed larger magnitudes of gp120 and V1V2 responses associated with higher BMI: *P* values comparing different BMI subgroups were <0.001, 0.015, and 0.0063 across the panels of clade C V1V2, multi-clade V1V2, and gp120 antigens, respectively, presented in table S4. In contrast to males, female vaccine recipients in HVTN 097 had borderline significantly higher response magnitudes to clade C V1V2, multi-clade V1V2, and gp120 panels: *P* values were 0.046, 0.10, and 0.048, respectively. The median magnitude of the readout [net mean fluorescent intensity (MFI)] to the clade C V1V2 panel was 3969 [95% confidence interval (CI), 1911 to 5557] in females and 1938 (95% CI, 1122 to 2900) in males ($P = 0.046$). No significant difference was seen in the magnitude of responses based on age.

Similar to the cellular responses, the response rate and magnitude of IgG or IgG3 responses to gp120 and V1V2 declined significantly over time ($P < 0.001$ for TH023.AE, A244.AE, and CaseA2_V1V2.B) (Table 2); the median magnitude responses of IgG or IgG3 gp120, gp140, V1V2, or V3 antibodies at the durability time point (6 months after second ALVAC/AIDSVAX vaccination) varied from 0.20 to 5.4% of those at the peak time point (2 weeks after second ALVAC/AIDSVAX vaccination) across various antigens among participants who were positive responders at peak (Table 2). The considerable decline in IgG response over time was also observed in RV144 (*20*, *23*, *24*). A significant difference in decline was seen between the two studies with regard to IgG gp70 scaffolded clade B CaseA2_V1V2. The decrease in response rate from peak to the durability time point was greater in RV144 (from 97.3 to 7.9%) compared to HVTN 097 (from 96.8 to 19.1%) ($P = 0.043$), and the fold decline in response magnitude was also greater in RV144 among positive responders at the peak time point ($P = 0.0011$).

Differences in sample type, serum (used in HVTN097) versus plasma (used in RV144), for the binding antibody assays were previously assessed (*19*). Although serum antibody responses were of slightly higher magnitude (at most, 0.10 $\log_{10}$ net MFI) compared to plasma antibody responses to certain gp120 antigens, no significant differences were detected for antibodies recognizing V1V2 antigens between sample types. A sensitivity analysis with a mean location shift of $-0.10\log_{10}$ applied to the HVTN 097 responses led to almost identical results as what observed in the main analyses regarding the comparison with RV144; the conclusion regarding the comparison between the two studies remains the same (*19*).

Table 2. Durability of immune responses among vaccine recipients in the per-protocol cohort of HVTN 097. MFI-Blank, background-subtracted MFI, where background refers to a plate level control (i.e., a blank well run on each plate).

Measure*	Peak time point (V14†) response rate	Durability time point (V17‡) response rate	McNemar's test *P* value V14 versus V17 (response rate)	Peak time point (V14) median magnitude (among V14 responders)	Durability time point (V17) median magnitude (among V14 responders)	Durability time point median/ peak time point median	Wilcoxon signed-rank test *P* value V14 versus V17 (magnitude)
$CD4^+$ Env.92TH023.AE§	70.8%	36.1%	<0.001	0.15% T cells expr. IL-2/IFN-γ	0.07% T cells expr. IL-2/IFN-γ	46.7%	<0.001
BAMA IgG 92TH023_D11gp120.AE	100%	51.5%	<0.001	17713.4 MFI-Blank	176.1 MFI-Blank	1.0%	<0.001
BAMA IgG A244 D11gp120_avi.AE	100%	98.5%	1	20274.8 MFI-Blank	1098.8 MFI-Blank	5.4%	<0.001
BAMA IgG Con 6 gp120/B	100%	62.7%	<0.001	15022.3 MFI-Blank	372.4 MFI-Blank	2.5%	<0.001
BAMA IgG MN gp120 gDneg/293F.B	100%	20.9%	<0.001	9507.4 MFI-Blank	208.2 MFI-Blank	2.2%	<0.001
BAMA IgG con_env03 140 CF.A1	100%	7.0%	<0.001	1453 MFI-Blank	17.9 MFI-Blank	1.2%	<0.001
BAMA IgG 01_con_env03 gp140CF_avi.AE	100%	67.2%	<0.001	14395.1 MFI-Blank	251.5 MFI-Blank	1.8%	<0.001
BAMA IgG Con S gp140 CFI	100%	33.3%	<0.001	17069.3 MFI-Blank	449 MFI-Blank	2.6%	<0.001
BAMA IgG A244 V1V2 Tags/293F.AE	100%	62.7%	<0.001	25793.7 MFI-Blank	680.5 MFI-Blank	2.6%	<0.001
BAMA IgG CaseA2_gp70_V1V2.B	96.8%	19.1%	<0.001	4145.6 MFI-Blank	23.5 MFI-Blank	0.6%	<0.001
BAMA IgG3 92TH023 gp120.AE	66.7%	1.4%	<0.001	475.8 MFI-Blank	1.8 MFI-Blank	0.4%	<0.001
BAMA IgG3 A244 D11gp120_avi.AE	72.2%	2.8%	<0.001	412 MFI-Blank	5.1 MFI-Blank	1.2%	<0.001
BAMA IgG3 Con 6 gp120/B	62.5%	0%	<0.001	246.9 MFI-Blank	4.1 MFI-Blank	1.7%	<0.001
BAMA IgG3 MN gp120 gDneg/293F.B	70.8%	1.4%	<0.001	741.4 MFI-Blank	2.8 MFI-Blank	0.4%	<0.001
BAMA IgG3 con_env03 140 CF.A1	9.7%	0%	0.016	172 MFI-Blank	3.8 MFI-Blank	2.2%	0.016
BAMA IgG3 01_con_env03 gp140CF_avi.AE	45.8%	1.4%	<0.001	295 MFI-Blank	3.6 MFI-Blank	1.2%	<0.001
BAMA IgG3 Con S gp140 CFI	75.0%	0%	<0.001	348.7 MFI-Blank	2.1 MFI-Blank	0.6%	<0.001
BAMA IgG3 A244 V1V2 Tags/293F.AE	88.9%	4.2%	<0.001	1464.8 MFI-Blank	6.5 MFI-Blank	0.4%	<0.001
BAMA IgG3 1086 V2 tags/293F.C	29.2%	1.4%	<0.001	241.1 MFI-Blank	9.2 MFI-Blank	3.8%	<0.001
BAMA IgG3 1086_V1V2_Tags.C	72.2%	1.4%	<0.001	727 MFI-Blank	6.6 MFI-Blank	0.9%	<0.001

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Measure*	Peak time point (V14†) response rate	Durability time point (V17‡) response rate	McNemar's test *P* value V14 versus V17 (response rate)	Peak time point (V14) median magnitude (among V14 responders)	Durability time point (V17) median magnitude (among V14 responders)	Durability time point median/ peak time point median	Wilcoxon signed-rank test *P* value V14 versus V17 (magnitude)
BAMA IgG3 CaseA2_gp70_ V1V2.B	28.6%	0%	<0.001	400.9 MFI-Blank	2.1 MFI-Blank	0.5%	<0.001
BAMA IgG3 MN V3 gp70.B	74.3%	0%	<0.001	491.8 MFI-Blank	1 MFI-Blank	0.2%	<0.001
BAMA IgG3 gp41	5.6%	5.6%	1	286.6 MFI-Blank	62 MFI-Blank	21.6%	0.125
BAMA IgG3 p24	70.8%	33.8%	<0.001	3013.3 MFI-Blank	177 MFI-Blank	5.9%	<0.001

*The clade of the antigen is indicated by the last letter(s) of the name (e.g., 92TH023_D11gp120.AE is clade AE). †Visit 14 (V14) corresponds to the peak immunogenicity time point (2 weeks after second ALVAC/AIDSVAX vaccination). ‡Visit 17 (V17) corresponds to the durability immunogenicity time point (6 months after second ALVAC/AIDSVAX vaccination). §This analysis uses 12-color flow cytometric panel originally used for the protocol, whereas the comparisons with RV144 elsewhere in this paper used an updated 16-color panel to match contemporaneous RV144 sample studies.

ADCC, ADCP, and neutralization

One of the aspects of our study was the examination of both ADCC and antibody-dependent cellular phagocytosis (ADCP) immune responses after vaccination. Vaccine-mediated protection may be achieved through the elicitation of polyfunctional immune responses, including ADCC and ADCP, aimed at the lysis or phagocytosis of target cells, respectively. The partial efficacy of RV144 has been correlated with ADCC mediated via the induction of antibodies that targeted the V1V2 region of the HIV-1 Env and has led to speculation that ADCC and other antibody-dependent cellular effector functions, such as ADCP, might provide a role in preventing mucosal acquisition of HIV-1, with ADCP acting through engagement on monocytes, macrophages, and dendritic cells, leading to phagocytosis of the opsonized virus and infected cells (*25*). In addition, both of these functional antibody responses have been reported to be associated with protection in nonhuman primate (NHP) studies (*26*, *27*). The response rate for ADCC antibodies as measured by the granzyme B assay using recombinant gp120-coated target cells was higher in HVTN 097 than in RV144: 72.6% (53 of 73) versus 58.5% (114 of 195) ($P = 0.04$) (Fig. 6A). This assay was chosen over the IFN-γ assay, which does not assess cell-mediated toxicity directly. Granzyme B is a key mediator of target cell death; therefore, release of granzyme B may be a more specific indicator of ADCC activity. We also evaluated the magnitude of the ADCC responses as area under the curve (AUC), and we observed similar ranges (7.17 to 62.46 for HVTN 097 and 4.24 to 62.76 for RV144) among the positive responders ($P = 0.45$) (Fig. 6A). Vaccine-elicited antibodies were also functional for ADCP activity (Fig. 6B). At the peak time point (2 weeks after second ALVAC/AIDSVAX vaccination), 100% of vaccine recipients had a positive ADCP response to HIV-1 Env (1086_gp140) compared to 0% of placebo recipients ($P < 0.001$). Among the vaccinees with positive responses, the range of mean phagocytosis score was between 7.0 and 26.9 for 1086_gp140 and was also significantly different between vaccine and placebo recipients ($P < 0.001$). The prevalence of ADCP antibodies to clade C gp140 immunogens was 100 and 77% to clade C V1V2 in HVTN 097.

Neutralizing antibody activity at the peak time point (2 weeks after second ALVAC/AIDSVAX vaccination) was seen only to viruses with a highly sensitive tier 1A neutralization phenotype (MN.3.B, TH023.6.AE, MW965.26.C, and SF162.LS.B) (fig. S4). Similar to the other cellular and humoral responses described above, the magnitude and breadth of neutralization of tier 1A viruses was higher in HVTN 097 vaccine recipients (mean AUC = 1.74) than in RV144 (mean AUC = 1.45) ($P < 0.001$; Fig. 6C). In particular, among positive responders, the magnitude was significantly higher for each of MN.3.B ($P < 0.001$), TH023.6.AE ($P < 0.001$), MW965.26.C ($P < 0.001$), and SF162.LS.B ($P = 0.022$) (table S3 and fig. S8). No neutralizing activity was detected against a global panel of five heterologous tier 2 CRF01_AE circulating strains (fig. S4B), suggesting that any protection this vaccine affords is not due to broadly reactive tier 2 HIV-1 neutralization.

Interaction between $CD4^+$ T cells expressing CD40L and antibody responses

Because of the major role of CD40L in providing T cell help to B cells, including B cell proliferation, isotype switching, and memory B cell function (*28*), we separately analyzed the association between percent of T cells expressing CD40L and binding antibody responses in RV144 and HVTN 097. As seen in Fig. 7, there was a weak association between $CD4^+$ Env-specific T cells to 92TH023-Env and a variety of binding antibodies. This included IgG and IgG3 to gp120 antigens (Fig. 7, A and B) and IgG and IgG3 AUC to V1V2 antigens from a variety of HIV-1 strains (Fig. 7, C and D).

Principal components analyses

Principal components analyses (PCA) revealed that HVTN 097 and RV144 vaccine recipients displayed overall similar immunological profiles, as seen by the mixture of individual responses by trial in Fig. 8A, with the variability in immune responses explained primarily by IgG binding antibodies and Env-specific $CD4^+$ T cells (both highly correlated with the first principal component that explains 50.2% variability of the immunological profile) (Fig. 8, A and B). Although CD40L responses to Env were related to binding antibody, Fig. 8B illustrates that T cell polyfunctionality per se was not well correlated with the antibody immune responses, indicating the importance of evaluating the specific cytokine expression patterns of the T cells to each vaccine regimen.

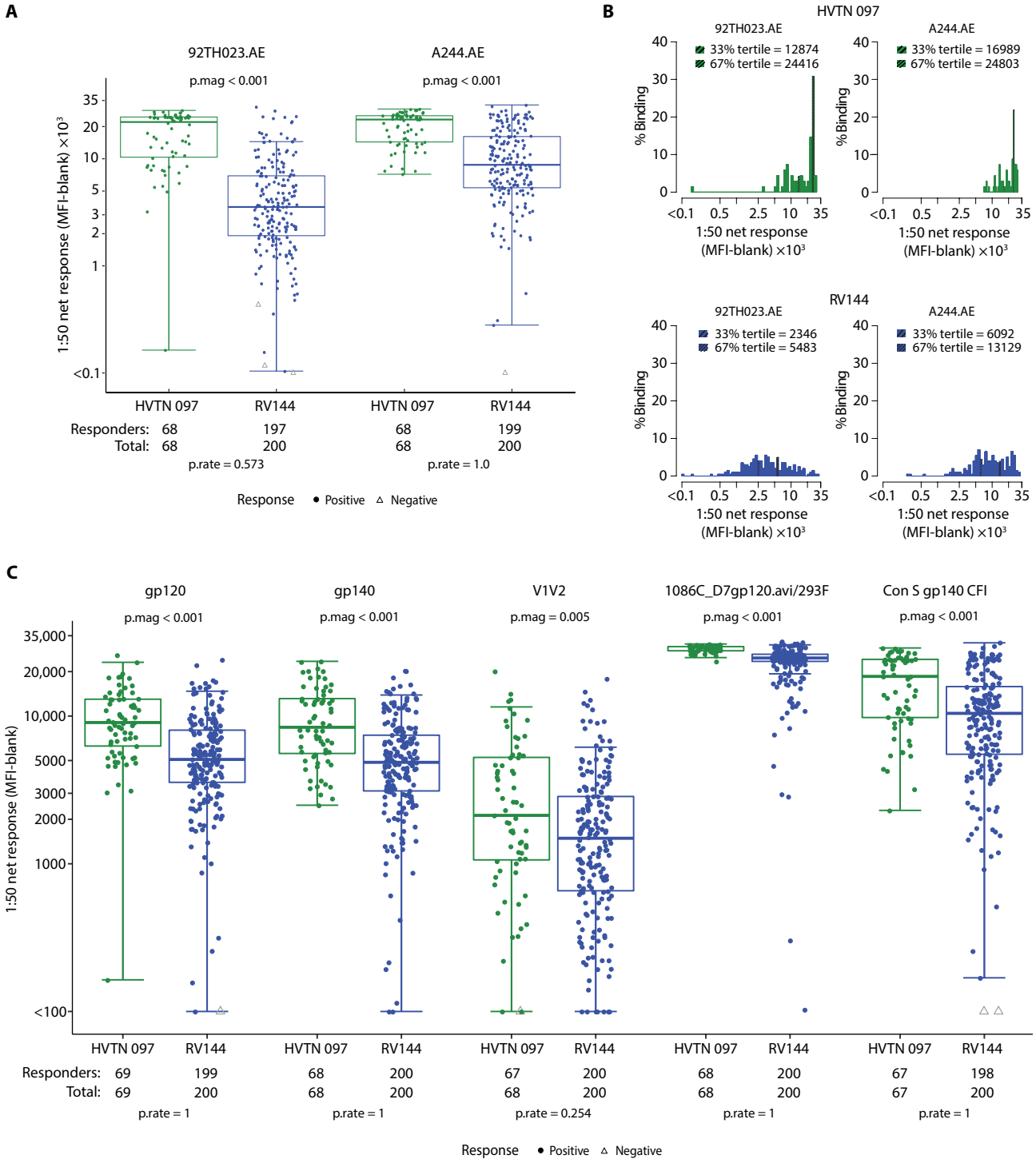


Fig. 4. IgG binding antibody responses in vaccine recipients to Env gp120 vaccine-insert antigens in the per-protocol cohorts of HVTN 097 and RV144 at the peak immunogenicity time point. IgG BAMA responses to Env gp120 vaccine-insert antigens (92TH023.AE and A244.AE) at the 1:50 dilution among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 2 weeks after the second ALVAC/AIDSVAX vaccination are shown by boxplot (**A**) and histogram (**B**). The net response magnitude (MFI-blank) is background-subtracted mean fluorescent intensity (MFI), where background refers to a plate level control (i.e., a blank well run on each plate). A post-enrollment sample is considered to be positive if the net magnitude is greater than or equal to an antigen-specific cutoff, the net magnitude is more than three times the baseline net magnitude, and the MFI is more than three times the baseline MFI. Fisher's exact test *P* values comparing response rates (p.rate) and Wilcoxon rank sum test *P* values comparing magnitudes (p.mag) among positive responders between HVTN 097 and RV144 vaccine recipients are provided in (A). (**C**) Geometric mean IgG binding antibody responses among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 at the peak immunogenicity time point. Fisher's exact test *P* values comparing response rates (p.rate) and Wilcoxon rank sum test *P* values comparing magnitudes (p.mag) among positive responders between HVTN 097 and RV144 vaccine recipients are provided. Boxplots are based on positive responders only represented by the green and blue circles for HVTN 097 and RV144, respectively; negative responders are shown as gray triangles.

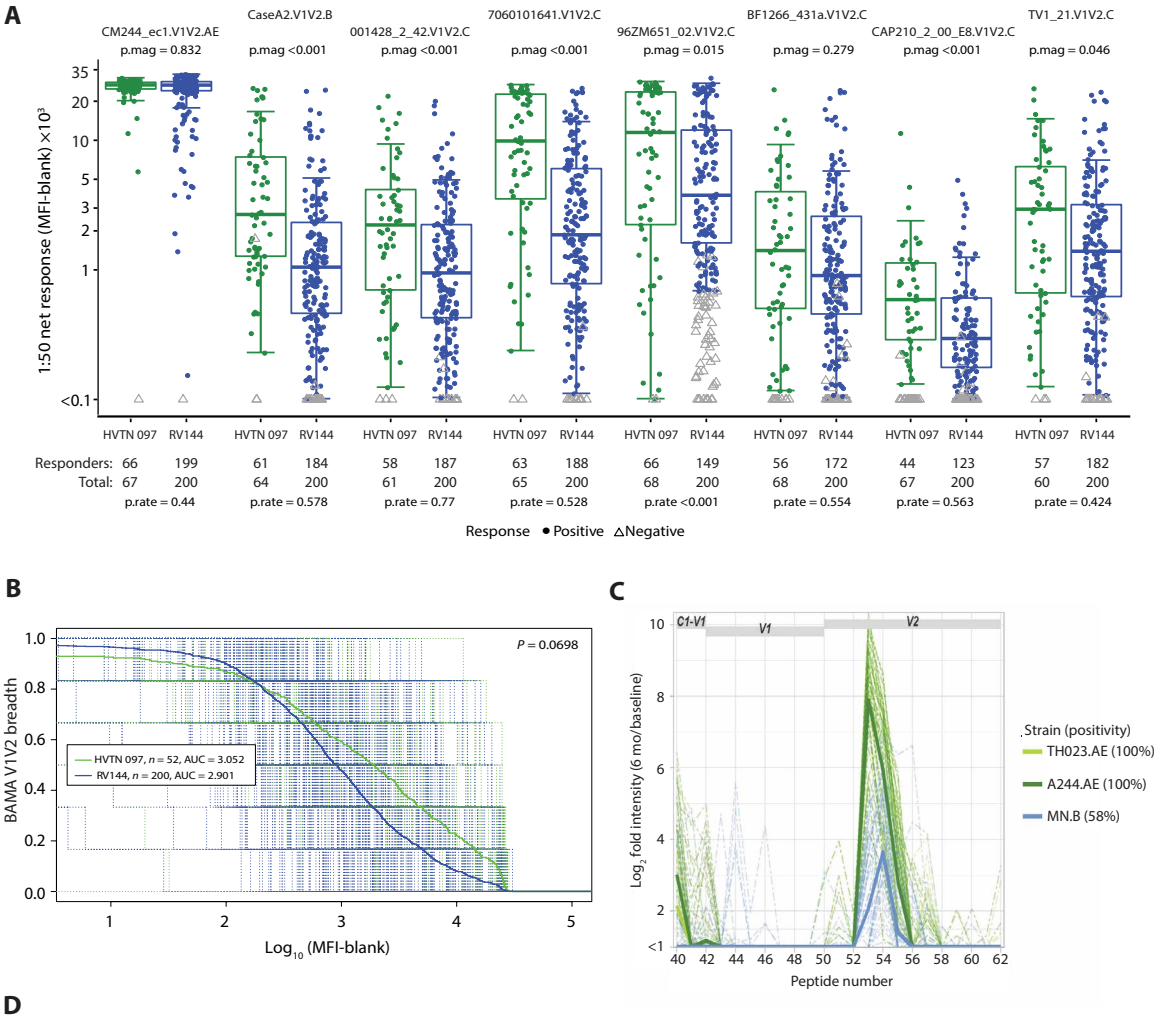


Fig. 5. IgG and IgG3 binding antibody responses to V1V2 antigens and magnitude-breadth plot of IgG binding antibody responses to clade C V1V2 antigens among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 at the peak immunogenicity time point. (**A**) IgG binding antibody responses to V1V2 antigens among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 2 weeks after the second ALVAC/AIDSVAX vaccination. (**B**) Magnitude-breadth plot of IgG binding antibody responses to clade C V1V2 antigens among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 2 weeks after the second ALVAC/AIDSVAX vaccination. Solid curves are average breadth across individuals for HVTN 097 and RV144 vaccine recipients, with breadth defined by the proportion of antigens in the panel with $\log_{10}$ (MFI-blank) greater than the threshold on the *x* axis. Clade C V1V2 antigens = gp70-001428_2_42 V1V2.C, gp70–7060101641 V1V2.C, gp70-96ZM651_02 V1v2.C, gp70-BF1266_431a_V1V2.C, gp70-CAP210_2_00_E8 V1V2.C, and gp70-TV1_21 V1V2.C. (**C**) Binding to peptides in V1V2 region of three vaccine strains. Magnitude of binding to overlapping peptides in V1V2 region of vaccine strains by serum IgG at 2 weeks after the second ALVAC/AIDSVAX vaccination. Thin lines represent individual participants. Thick lines represent weighted means. (**D**) IgG3 binding antibody responses to V1V2 antigens among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 2 weeks after the second ALVAC/AIDSVAX vaccination. Boxplots are based on positive responders only, with negative responders shown as gray triangles with positive response rates above the boxes. Fisher's exact test *P* values comparing response rates (p.rate) and Wilcoxon rank sum test *P* values comparing magnitudes (p.mag) among positive responders between HVTN 097 and RV144 vaccine recipients are provided.

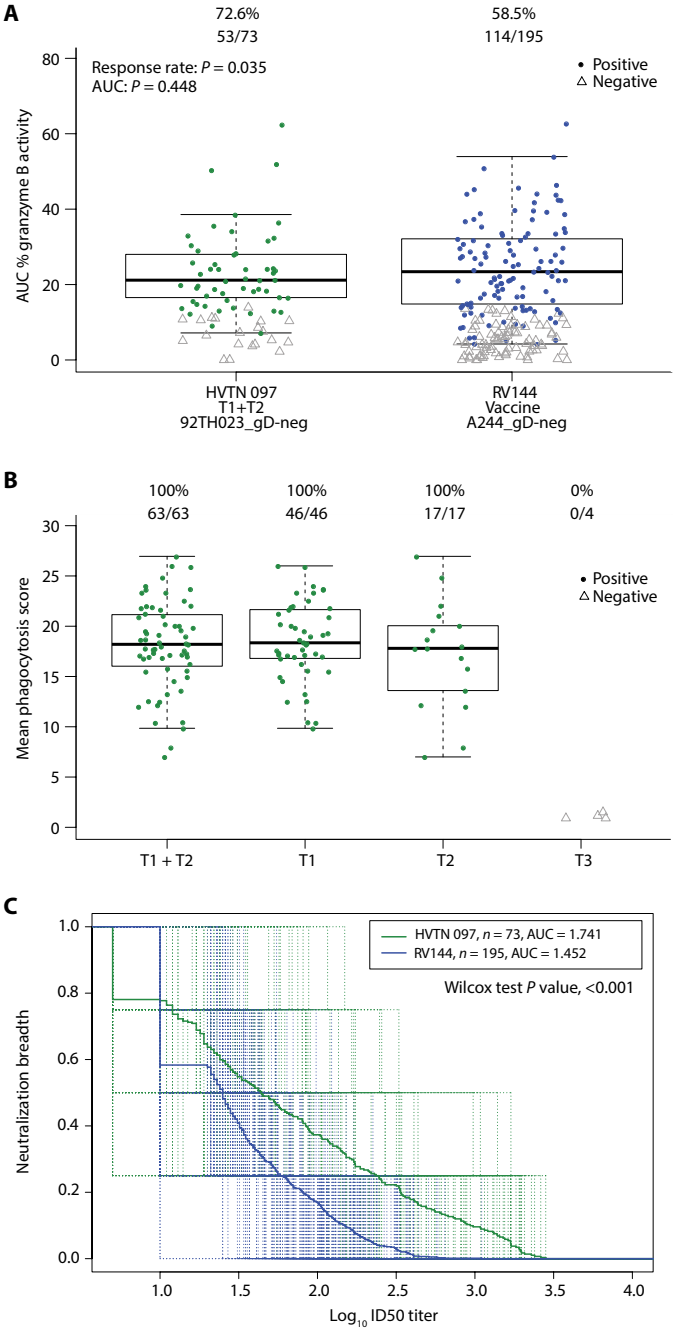


Fig. 6. Antibody responses in vaccine recipients in HVTN 097 and RV144. (**A**) ADCC responses were evaluated against subtype AE 92TH023_gD-neg and A244_gD-neg recombinant gp120–coated CEM.NKR$_{CCR5}$ target cells for the HVTN 097 ($n = 73$) and RV144 ($n = 195$) samples collected at the peak immunogenicity time point (2 weeks after the second ALVAC/AIDSVAX vaccination), as indicated on the *x* axis. Peripheral blood mononuclear cells from one normal healthy HIV-seronegative donor were used as source of effector cells. The *y* axis represents the values of the area under the curve (AUC) to represent the magnitude of responses. Boxplots are based on positive responders only represented by the green and blue circles for HVTN 097 and RV144, respectively; negative responders are shown as gray triangles. Response rates and number of responders over total are reported above each group boxplot. *P* value comparing response rates among positive responders between HVTN 097 and RV144 vaccine recipients is provided. (**B**) IgG-mediated ADCP was tested at study baseline and 2 weeks after the second ALVAC/AIDSVAX vaccination for a subset of per-protocol participants, with 63 vaccine recipients (46 in group T1 and 17 in group T2) and 5 placebo recipients. ADCP score using the human THP-1 cell line is shown. (**C**) Neutralizing antibody responses (magnitude-breadth curves) to tier 1 viruses among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 2 weeks after the second ALVAC/AIDSVAX vaccination. Solid curves are average breadth across individuals for HVTN 097 and RV144 vaccine recipients, with breadth defined by the proportion of antigens in the panel with $\log_{10}$ ID50 titer greater than the threshold on the *x* axis.

DISCUSSION

Findings in this study demonstrate that the immune responses associated with reduced risk of HIV-1 infection in the RV144 trial can be elicited, and often at greater frequency and magnitude, among HIV-1–seronegative South Africans. This was seen, irrespective of sex, age, and locale, in both Env-specific antibody and CD4$^+$ T cell responses. In addition, the RV144 vaccine regimen, although designed for the Southeast Asian clade A/E epidemic, elicited substantial cross-clade immune responses to antibodies and T cell antigens derived from the predominantly clade C epidemic in sub-Saharan Africa, indicative of this regimen's potential for global coverage. We demonstrate several interesting functional antibody responses associated with protection of infection in both NHP and human vaccine trials, including high frequencies of ADCP, ADCC, and CD40L$^+$CD4$^+$ T cell responses to HIV-1 Env.

Our data are encouraging because several other HIV vaccine studies have demonstrated a differential effect of sex and BMI on vaccine-induced immune responses. In South Africa, the HVTN 503/Phambili study demonstrated an inverse relationship of MRK Ad5 HIV-1 *gag/pol/nef* vaccine–induced CD4$^+$ T cell immune response with BMI: Overweight and obese participants had more muted responses compared to participants with low/normal BMI (*21*). In contrast, there were no negative effects of BMI on vaccine-induced CD4$^+$ T cell immune responses or on IgG binding antibody responses in HVTN 097. As BMI data were not collected in RV144, a limitation of our study was our inability to compare the impact of BMI on immune responses across the two studies. Of the 100 participants enrolled in HVTN 097, only 15% of the cohort had a BMI > 31, reducing the generalizability of our finding. Given the regional differences, BMI assessments in RV144 would have provided valuable insights in the role that body mass plays on vaccine-induced immune responses.

Notably, HVTN 097 showed cellular responses to vaccination to be similar in both sexes. There have been inconsistent results for CD4$^+$ T cell responses by sex in studies of a recombinant pox vector, NYVAC-C, where some studies have demonstrated no differences by sex, whereas in another study, females were more likely to be responders, as compared to males (*21*).

The observation that South Africans had stronger immune responses than Thais may be due to an interplay between race, ethnicity, genetic factors, pathogen exposure, the microbiome, or factors such as smoking or alcohol use that have affected immune responses to other vaccines (*29*, *30*). A further limitation of our study was the limited baseline demographic information we had for both populations that may have assisted us further in understanding the differences we saw in vaccine-induced immune responses. The AIDSVAX B/E protein boost immunogen was identical between the two studies (same lot). Although the manufacturing lot of the ALVAC prime was different, the release assays were similar, making manufacturing in our opinion an unlikely explanation for the differences observed here.

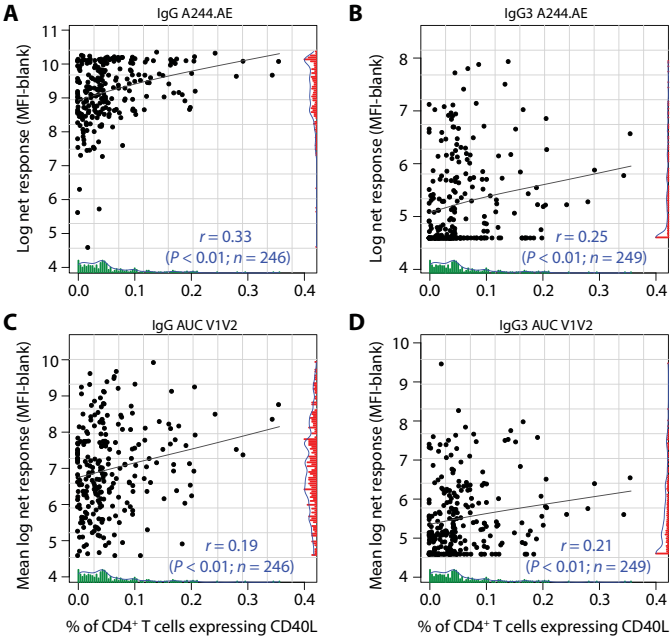


Fig. 7. Association between percent of CD4$^+$ T cells expressing CD40L reactive to HIV-1 Env vaccine strain 92TH023 and binding antibodies at the peak immunogenicity time point. (**A**) IgG response to A244.AE gp120. (**B**) IgG3 response to A244.AE gp120. (**C**) IgG response (AUC) to clade C V1V2 panel. (**D**) IgG3 response (AUC) to clade C V1V2 panel. *X* axis is percent of CD4$^+$ T cells expressing CD40L reactive to 92TH023-ENV, and a histogram of that distribution is shown in green. Along the *y* axis, a histogram of the corresponding *y* variable is shown in red.

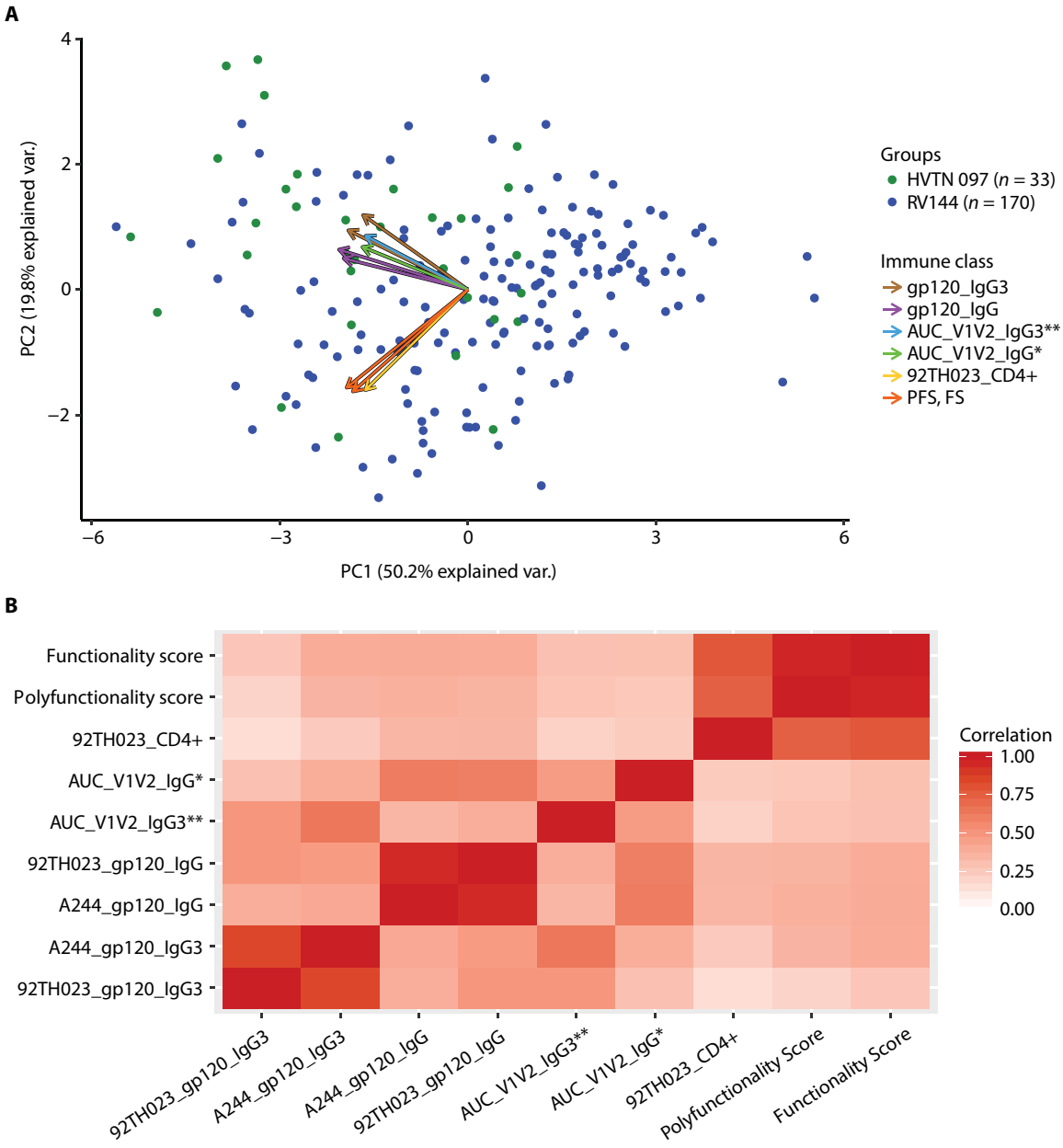
Although binding antibody responses measured in serum are slightly higher than those measured in plasma (*31*), our detailed statistical analyses indicate that this did not account for the differences observed in this study.

Different genetic background may explain the differences in immune response seen between these two populations: Race has been associated with higher measles vaccine–induced antibody and cell-mediated immune responses in African Americans relative to Caucasians (including Hispanics) (*32*). In contrast, Caucasians have demonstrated higher vaccinia-specific cytokine responses after primary small pox vaccination as compared to African Americans and Hispanics (*33*). Differing genetic background, most notably at key polymorphic loci such as the HLA region, may account for the variation in vaccine-induced immune responses, especially the variances in HLA class II genes between individuals participating in the RV144 trial in Thailand and the HVTN 097 trial in RSA. In RV144, clear differences were seen in correlation between vaccine-induced immune responses and efficacy among individuals having DQB1*06 and DPB1*13 alleles: Env-specific IgA antibodies were associated with increased risk of HIV acquisition only among individuals with DQB1*06, and IgG antibodies to Env amino acid positions 120 to 204 were higher and associated with decreased risk of HIV acquisition only among individuals with DPB1*13 (*17*). There is a higher frequency of DQB1*06 in South Africans (32.4%), as compared to Thais (10.3%) (*34*, *35*); however, the overall frequency of the DPB1*13 allele among South Africans requires further study. In HVTN 097, the allele prevalence was found to be 31.8% for DQB1*06 and 4.7% for DPB1*13. Given the limitation of our sample size, the role of HLA II DQB1*06 and DPB1*13 in vaccine-induced immune response and efficacy will be of importance to evaluate in the phase 2b/3 study HVTN 702 testing a clade C adapted RV144 vaccine regimen in South Africa. Little is known about the association between class II alleles and CD40L interacting in risk of HIV infection. Cross-linking affinities to the cognate antigen have been shown to influence B cell proliferation and isotype switching. The variation in host responses may affect the efficacy of an HIV vaccine, and further exploration is required to understand the interplay between genetic background and vaccine-induced immune responses.

As Fc receptors play an important role in antibody-mediated action of HIV-specific antibodies, polymorphic allelic forms of FcγRIIA and FcγRIIIA with a higher affinity could be associated with more efficient vaccine response. A conclusive result as to the relative role of polymorphic allelic variants of these receptors is still, however, elusive, and the variation of Fcγ receptor genes in various populations remains to be determined. This issue is pertinent to the ADCP and ADCC responses elicited by the HVTN 097 vaccine regimen: ADCP responses have recently been reported to be an important correlate of protection in NHP models of an Ad26 gp120 vaccine regimen (*36*) and, in follow-up studies, were associated with the V1V2 IgG and IgG3 correlate of risk in RV144 (*13*, *37*). The finding here that HVTN 097 elicited a 100% prevalence of ADCP to gp140 with a significant range in titer supports the potential utility as a correlate of risk in the ongoing HVTN 702 efficacy trial. Although ADCC responses using the granzyme B assay were of somewhat lower prevalence (72%), the importance of its role as a functional marker of non-neutralizing antibodies in vaccine protection has been suggested in several other settings (*38*). The use of gp120-coated cells for ADCC measurement does not have the same relevance as assays based on infected cells, but we consider this approach to be reasonable in the context of high-throughput immune assessments and because results from these assays have correlated with control of virus replication and infection in preclinical trials and with protection in mother-to-child-transmission (*25*, *39*). The variability observed in the ADCC responses based upon the HIV-1 strain used in these assays highlights the need for development of a defined panel of antigens to more systematically define ADCC responses after vaccination, especially in the V2 region of HIV-1 to evaluate ADCC as a functional correlate to the binding antibodies that were associated with vaccine efficacy in RV144.

Although the regimen we used in the study could be considered first generation, a strength of our study was the assessment of immune responses using comprehensive laboratory techniques combined with new analytical approaches. These included detailed evaluation of the cytokine patterns of HIV-1–specific CD4$^+$ and CD8$^+$ T cells, as well as an extensive evaluation of cross-clade antibody responses to the HIV-1 Env. Our findings indicate that CD4$^+$ T cell responses elicited by the RV144 regimen were polyfunctional, with IFN-γ, CD40L, IL-2, and TNF-α single or co-expressing subsets being the most common. The degree of functionality and polyfunctionality has previously been associated with reduction in HIV-1 acquisition in RV144 (*8*). Although polyfunctionality has been associated with greater in vivo function of CD4$^+$ and CD8$^+$ T cells, our data indicate that, not unexpectedly, the individual function matters. The association between Env-specific CD4$^+$ T cells expressing CD40L and antibody responses has potential mechanistic underpinnings that should be pursued as a strategy to improve both the magnitude and durability issues that HIV vaccines need to overcome.

Perhaps the most important implication of our study is that the vaccine regimen used in RV144 might be effective in regions of the



*AUC_v1v2_IgG = mean response to gp70-001428_2_42 V1V2.C, gp70-7060101641 V1V2.C, gp70-96ZM651_02 V1V2.C, gp70-BF1266_431a_V1V2.C, gp70-CAP210_2_00_E8 V1V2.C, gp70-TV1_21 V1V2.C.
**AUC_v1v2_IgG3 = mean response to A244 V1V2 Tags/293F.AE, 1086_V1V2 Tags.C, CaseA2_gp70_V1V2.B.

Fig. 8. Multi-assay PCA. (**A**) Biplot and (**B**) Spearman correlation heatmap for vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 at the peak immunogenicity time point (2 weeks after second ALVAC/AIDSVAX vaccination). In (A), the *x* axis is the value from the first principal component, and the *y* axis is the second principal component, where each axis label includes the percentage of variation in the total set of readouts captured by the principal component. Points on the plot represent the values of the principal components of each observation. Points that are close together correspond to observations that have similar values on the components displayed in the plot. The top axis is the first principal component loadings, and the right axis is the second principal component loadings, where loadings are the weights by which each original immunogenicity endpoint score should be multiplied to get the value of the first or second principal component. An arrow (vector) is drawn for each immunogenicity endpoint from the origin to the point defined by its first two principal component loadings. Vectors that point in the same direction correspond to endpoints that have similar response profiles on the basis of the first two principal components. The points that project furthest in the direction in which the vector points are the observations that have the most weight of whatever the endpoint measures. Those points that project at the other end have the least weight of whatever the endpoint measures. The angle between two arrows conveys information about the correlation of the two endpoint scores, with a 0° angle denoting perfect correlation and a 90° angle denoting no correlation. In (A), gp120_IgG3 stands for 92TH023_gp120_IgG3 and A244_gp120_IgG3, gp120_IgG stands for 92TH023_gp120_IgG and A244_gp120_IgG, and PFS(FS) stands for polyfunctionality score (functionality score).

world where clade A, AE, E, B, and C infections predominate. Our data suggest that the breadth of immune responses elicited by this vaccine regimen may allow for vaccine protection that could extend beyond the clade used for immunogen development, and potentially function as a more global vaccine. The RV144 cohort used for our analysis selected in 2015 was generated for our companion study HVTN 100 (*31*). HVTN 100 had similar enrollment criteria and demographics as HVTN 097, yet the immune profile of HVTN 097 is distinct from HVTN 100, with HVTN 100 inducing higher $CD4^+$ T cell responses and gp120-binding antibody responses, but a lower anti-V1V2 IgG response rate than seen in both RV144 and HVTN 097 (*31*). Thus, our analyses provide a comparison of the immunological correlates of infection risk with the RV144 regimen in South Africans, and a direct comparison to HVTN 100, which was also conducted in South Africa (*19*). These differences in immune responses using the same vector backbone with a different protein boost indicate the potential importance of the immunogen Env sequence itself in defining the immune response to vaccination. The immune protection of the RV144/HVTN 097 regimen appears to be multicomponent, i.e., a combination of $CD4^+$ T cell as well as non-neutralizing antibody responses and hence detailed evaluation and studies of these components need to be performed in the context of efficacy trials. These evaluations should include both clade-specific and cross-clade–specific immune assays.

In both studies, the T cell and antibody responses waned over time, suggesting the utility of additional boosts. A study conducted in South Africa evaluating a clade C DNA/MVA vaccine regimen (HVTN 073e) and a RV144 follow-up study (RV305) demonstrated the value of a protein boost given more than 2 years after the final boost. In HVTN 073e, boosting with a subtype C gp140 enhanced the magnitude of binding and neutralizing antibody responses and Env-specific $CD4^+$ T cell responses (*40*). In RV305, the delayed boost increased humoral and cellular immune responses (*41*). The addition of a boost may be critical to further mature protective immunity and sustain vaccine efficacy until approaches achieving greater durability can be found.

In summary, the evaluation of the RV144 Thai regimen in South Africa has been critical to our iterative approach to modify and adapt this regimen to a clade C–endemic region. An effective HIV vaccine will only be garnered by the continued rigorous evaluation of vaccine platforms and immunogens. These data may indicate that cross-clade immune responses, especially to non-neutralizing epitopes correlated with decreased HIV-1 risk, can be achieved for a globally effective vaccine by using unique HIV Env strains.

MATERIALS AND METHODS

Study design

HVTN 097 was a phase 1b randomized, double-blind, placebo-controlled clinical trial to assess the safety and immunogenicity of two administrations of ALVAC-HIV followed by two co-administrations of ALVAC-HIV and AIDSVAX B/E. Participants were enrolled at three sites in South Africa: Cape Town, Western Cape province (HIV prevalence, 5.0%); Klerksdorp, North West province (HIV prevalence, 13.3%); and Soweto, Gauteng province (HIV prevalence, 12.4%) (*42*). The study was approved by the University of the Witwatersrand Human Research Ethics Committee for Klerksdorp and Soweto sites and by the University of Cape Town Ethics Committee for the Cape Town site. The trial was registered with the U.S. National Institutes of Health (NIH) Clinical Trials Registry (ClinicalTrials.gov NCT02109354) and the South African National Clinical Trials Registry (SANCTR number: DOH-27-0313-4201). The CONSORT diagram is shown in Fig. 1.

The randomization sequence was obtained by computer-generated random numbers provided to each site through a web-based randomization system. Randomization was stratified by site and done in blocks to ensure balance across arms.

Participants and site staff were blinded as to participant group assignments. Site pharmacists were not blinded to ensure proper handling and dispensing of study product. Overlays were applied to all syringes before delivery to the clinic staff to ensure allocation concealment. Division of AIDS protocol pharmacists, contract monitors, data management center staff, and the safety monitoring board were unblinded to ensure proper trial conduct and safety review.

The primary objectives were to evaluate the safety and immunogenicity of the RV144 vaccine regimen in healthy HIV-1–uninfected adults in South Africa. Primary safety endpoints included local and systemic reactogenicity signs and symptoms through the vaccination schedule.

Primary immunogenicity endpoints included the frequency and magnitude of IgG and IgG3 antibody binding, measured by the HIV-1 BAMA on serum specimens obtained at baseline, at a peak time point (2 weeks after second ALVAC/AIDSVAX vaccination), and a durability time point (6 months after second ALVAC/AIDSVAX vaccination), and the response rates and magnitudes of $CD4^+$ and $CD8^+$ T cell responses at the baseline, peak, and durability time points, as measured by intracellular cytokine staining.

Study population

Volunteers were eligible for enrollment if they were 18 to 40 years old, able to give voluntary written informed consent, found to be healthy, HIV uninfected, and at low risk for HIV acquisition and had not previously received an experimental vaccine (HIV vaccine or unlicensed non-HIV vaccine). Low risk was defined as being sexually abstinent, or in a mutually monogamous relationship with a partner of known HIV-uninfected status, or having one partner believed to be HIV-uninfected with whom he/she regularly used condoms for vaginal or anal intercourse, as well as not having a history of newly acquired sexually transmitted infections during the 12 months before enrollment. Females were required to be on contraception, nonpregnant, and not lactating. All trial participants gave written informed consent.

Study products

Investigational products included ALVAC-HIV (vCP1521) manufactured by IDT Biologika, Germany, for Sanofi Pasteur and AIDSVAX B/E manufactured by Global Solutions for Infectious Diseases. ALVAC-HIV (vCP1521) is a preparation of a live attenuated recombinant canarypox-derived virus expressing gene products from the HIV-1 gp120 (92TH023/clade E), Gag (clade B), and Pro (clade B) coding sequences and cultured in chicken embryo fibroblast cells. The actual measured dose of ALVAC-HIV was 5.2×10^7 cell culture infectious doses, 50% endpoint ($CCID_{50}$) per dose. The placebo for ALVAC-HIV was a mixture of virus stabilizer and freeze drying medium reconstituted with sodium chloride (0.4% NaCl). AIDSVAX B/E is a bivalent HIV gp120 glycoprotein (B.MN and E.A244) produced in Chinese hamster ovary cells and absorbed onto 600 μg of aluminum hydroxide gel adjuvant. Each dose of AIDSVAX B/E was 1 ml (300 μg of each gp120 and 600 μg of total protein). The placebo

for AIDSVAX B/E was 0.9% sodium chloride. Participants were randomly assigned to receive placebo or ALVAC-HIV (vCP1521) at months 1 and 2 and ALVAC-HIV (vCP1521) plus AIDSVAX B/E at the peak immunogenicity time point (2 weeks after second ALVAC/AIDSVAX vaccination). Study products were administered by intramuscular injection.

Study procedures

Screening procedures included consent and assessment of understanding, medical history, physical examination, behavioral risk assessment, urine dipstick, pregnancy test, and blood collection for complete blood count, chemistry panel, hepatitis B and C, syphilis, and immunogenicity assays.

At all visits, clinical assessments and risk reduction counseling were conducted. Participants were followed for 13.5 months after enrollment.

Sample size

One hundred healthy participants were randomized in 3:1:1 ratio to group 1 (HIV vaccines + tetanus vaccine + hepatitis B vaccine: T1), group 2 (HIV vaccine only: T2), and placebo group (tetanus vaccine + hepatitis B vaccine: T3). The tetanus and hepatitis B immunizations were performed to evaluate any correlates of immune responses to HIV vaccine response. Given that there were no meaningful differences in HIV immune responses between HIV vaccinees with or without tetanus + hepatitis B vaccines (*43*), the analyses described in this paper are all associated with the responses to HIV-1 and based on pooling data from groups 1 and 2, henceforth referred to as the vaccine group or T1 + T2 (Fig. 1).

Comparisons to RV144

To optimize the comparability of the two studies, we evaluated cellular and humoral immunogenicity contemporaneously in the same laboratory from samples obtained prospectively in HVTN 097 and from archived samples in RV144 (called the 2015 Contemporaneous Cohort). All laboratory assays were performed blinded to the treatment group, and the methods have been validated, qualified, and used in HVTN 100 (*19*). Analyses were based on the per-protocol cohort of RV144, consisting of all participants who were HIV-1 uninfected at the fourth vaccination who received all four HIV vaccinations and did not have specified protocol violations. The timing of sampling for RV144 was exactly the same as in HVTN 097. Immune response data for Env-specific $CD4^+$ and $CD8^+$ T cell and antibody responses in RV144 originated from a subset selected in 2015 from the uninfected RV144 vaccine and placebo recipients, who were age- and sex frequency–matched to South African participants enrolled in HVTN 100 (*31*). These samples allowed contemporaneous evaluation of both T cell and antibody responses in HVTN 097 and RV144. Neutralization antibody response data in RV144 were from the uninfected RV144 vaccine and placebo recipients selected in 2010 for the immune correlates study, called the case-control study (Table 1) (*4*). There were no statistical differences with regard to treatment/placebo, age, or sex in the two cohorts.

Statistical analyses

Box plots and histograms graphically display distributions of immune responses to individual antigens at each given time point. The midline of the box denotes the median, and the ends of the box denote the 25th and 75th percentiles. The whiskers that extend from the top and bottom of the box extend to the most extreme data points that are no more than 1.5 times the interquartile range (i.e., height of the box) or, if no value meets this criterion, to the data extremes. Immune response was summarized by the percentage of participants who had a positive response ("positive response rate"), by the estimated pseudo-median titer (median if the distribution is symmetric) based on the Hodges-Lehmann estimator, and by geometric mean titer (GMT). The 95% CIs for positive response rate and for pseudo-median titer were computed on the basis of the Wilson method (*44*) and the algorithm described by Bauer (*45*). To describe the magnitude and breadth across a panel of antigens, the MB curve was used with the AUC computed as a summary measure (*46*). Antigen-specific T cell subsets were analyzed by COMPASS with FS and PFS defined above. COMPASS posterior probabilities and the correlations between pairs of immune responses were reported for HVTN 097 and RV144. PCA biplots were used to visualize the multivariate immune response data in HVTN 097 and RV144 (*47*).

For comparison of immune responses between two (or more) independent groups (e.g., vaccine groups in HVTN 097 and RV144 or BMI subgroups among HVTN 097 vaccine recipients), Fisher's exact test and the Wilcoxon rank sum test (or Kolmogorov-Smirnov test) were used for comparison of response rates and magnitudes among positive responders, respectively. For comparison of immune responses between two time points (peak versus durability), McNemar's test and the Wilcoxon signed rank test were used for comparison of response rate and magnitude among positive responders at peak time point, respectively.

All P values are two-sided, and significance is declared for $P < 0.05$. Statistical analyses were performed using SAS (version 9.4, SAS Institute) and R statistical software (version 2.15.1, R Foundation for Statistical Computing).

SUPPLEMENTARY MATERIALS

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Materials and Methods

Fig. S1. Reactogenicity bar charts for HVTN 097.

Fig. S2. IgG binding antibody responses to cross-clade V1V2 antigens among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 at the peak immunogenicity time point.

Fig. S3. IgG3 binding antibody responses to gp120 antigens among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 at the peak immunogenicity time point.

Fig. S4. Neutralizing antibody responses in the per-protocol cohorts of HVTN 097 and RV144 at the peak immunogenicity time point.

Table S1. Comparison of ICS (IL-2^+ and/or IFN-γ^+) $CD4^+$ and $CD8^+$ T cell responses to Env at the peak immunogenicity time point among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144.

Table S2. Details of the BAMA, ICS, neutralizing antibody, ADCC, and ADCP antigens including HIV-1 viral strain information.

Table S3. Response rates among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 for IgG and IgG3 binding antibody and neutralizing antibody responses at the peak immunogenicity time point.

Table S4. IgG binding antibody response among vaccine recipients in the per-protocol cohorts of HVTN 097 and RV144 at the peak immunogenicity time point by age, sex, and BMI.

References (*48*, *49*)

REFERENCES AND NOTES

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Immune correlates of the Thai RV144 HIV vaccine regimen in South Africa

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Taking RV144 beyond Thailand

The RV144 vaccine trial in Thailand is the only HIV vaccine to show efficacy against HIV infection to date. Gray *et al.* designed the HVTN 097 trial to test this regimen in South Africa, where clade C HIV circulates; this clade is heterologous to the vaccine antigens. They intently examined immune protective responses previously identified in the RV144 trial and found that the vaccine seemed to be even more immunogenic in South Africans. CD4+ T cell responses were stronger and more common in HVTN 097, and the magnitude of protective antibody responses was greater compared to RV144. Their results indicate that the RV144 regimen or others like it could be protective in areas where HIV is endemic.

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